FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 47-164

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 10/009301 Unassigned

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU00/00638	7 June 2000	7 June 1999

TITLE OF INVENTION

METHOD OF TREATING CARCINOMA USING ANTIBODY THERAPY AND AMELIORATING SIDE EFFECTS ASSOCATED WITH SUCH THERAPY

APPLICANT(S) FOR DO/EO/US

SMITH et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is attached hereto.
   - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 To 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information.

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unassigned 10/009301	PCT/AU00/00638	47-164

21. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY		
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):			
-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00			
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00			
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO $740.00			
-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00			
-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00			
ENTER APPROPRIATE BASIC FEE AMOUNT =	$	1040.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$	130.00	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE			
Total Claims	19	-20 =	0	X	$18.00	$	0.00
Independent Claims	3	-3 =	0	X	$84.00		0.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					$280.00	$	0.00

TOTAL OF ABOVE CALCULATIONS =	$	**1170.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.		0.00	
SUBTOTAL =	$	**1170.00**	
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +		0.00	
TOTAL NATIONAL FEE =	$	**1170.00**	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +	$	0.00	
Fee for Petition to Revive Unintentionally Abandoned Application ($1280.00 – Small Entity = $640.00)	$	0.00	
TOTAL FEES ENCLOSED =	$	**1170.00**	
Amount to be: refunded	$		
Charged	$		

a. ☒ A check in the amount of $1170.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☒ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Leonard C. Mitchard
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29,009 December 7, 2001
REGISTRATION NUMBER Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

SMITH et al

Atty. Ref.: 47-164

Serial No. Unassigned

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Examiner:

For: METHOD OF TREATING CARCINOMA USING ANTIBODY THERAPY AND AMELIORATING SIDE EFFECTS ASSOCIATED WITH SUCH THERAPY

* * * * * * * * * * *

December 7, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Please amend this application as follows:

IN THE CLAIMS

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

5. (Amended) A method as claimed in claim 1 in which the method comprises administering an H1 and H2 receptor antagonist.

6. (Amended) A method as claimed in claim 1 in which the H1 and/or H2 receptor antagonist is a non-specific antagonist.

7. (Amended) A method as claimed in claim 1 in which the Hi receptor antagonist is promethazine or a pharmaceutically acceptable salt thereof.

8. (Amended) A method as claimed in claim 1 in which the H2 receptor antagonist is ranitidine or a pharmaceutically acceptable salt thereof.

9. (Amended) A method as claimed in claim 3 in which the H1 and/or H2 receptor antagonist is administered to the subject prior to administration of 30.6 antibody.

11. (Amended) A method as claimed in claim 1 in which the H1 receptor antagonist is administered intramuscularly.

12. (Amended) A method as claimed in claim 1 in which the H2 receptor antagonist is administered intravenously,

13. (Amended) A method as claimed in claim 1 in which the H1 and/or H2 receptor antagonist is administered at a dosage level of around the maximum tolerated dose.

16. (Amended) A method as claimed in claim 15 in which the 30.6 antibody is a chimeric 30.6 antibody.

18. (Amended) A method as claimed in claim 3 in which the 30.6 antibody is a murine antibody.

19. (Amended) A method as claimed claim 3 in which the 30.6 antibody is a humanized antibody.

REMARKS

The above amendments have been made to place the application in a more traditional format. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made**."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

5. (Amended) A method as claimed in [any one of claims 1 to 4] claim 1 in which the method comprises administering an H1 and H2 receptor antagonist.

6. (Amended) A method as claimed in [any one of claims 1 to 5] claim 1 in which the H1 and/or H2 receptor antagonist is a non-specific antagonist.

7. (Amended) A method as claimed in [any one of claims 1 to 6] claim 1 in which the Hi receptor antagonist is promethazine or a pharmaceutically acceptable salt thereof.

8. (Amended) A method as claimed in [any one of claims 1 to 7] claim 1 in which the H2 receptor antagonist is ranitidine or a pharmaceutically acceptable salt thereof.

9. (Amended) A method as claimed in [any one of claims 3 to 8] claim 3 in which the H1 and/or H2 receptor antagonist is administered to the subject prior to administration of 30.6 antibody.

11. (Amended) A method as claimed in [any one of claims 1 to 10] claim 1 in which the H1 receptor antagonist is administered intramuscularly.

12. (Amended) A method as claimed in [any one of claims 1 to 11] claim 1 in which the H2 receptor antagonist is administered intravenously,

13. (Amended) A method as claimed in [any one of claims 1 to 12] claim 1 in which the H1 and/or H2 receptor antagonist is administered at a dosage level of around the maximum tolerated dose.

16. (Amended) A method as claimed in [any one of claims 3 to 15] claim 15 in which the 30.6 antibody is a chimeric 30.6 antibody.

18. (Amended) A method as claimed in [any one of claims 3 to 15] claim 3 in which the 30.6 antibody is a murine antibody.

19. (Amended) A method as claimed [any one of claims 3 to 15] claim 3 in which the 30.6 antibody is a humanized antibody.

METHOD OF TREATING CARCINOMA USING ANTIBODY THERAPY AND AMELIORATING SIDE EFFECTS ASSOCIATED WITH SUCH THERAPY

FIELD OF THE INVENTION

The present invention relates generally to a method of ameliorating or preventing temporal progression of burning cutaneous erythema. The present invention also relates to a method of treating carcinoma using antibody therapy. In particular the present invention relates to a method of ameliorating or preventing adverse side effects associated with such therapy.

BACKGROUND OF THE INVENTION

Over the last few years, a number of clinical studies have provided evidence that monoclonal antibodies are of value in the treatment of a variety of cancers, including breast, lymphoma and colorectal tumors [1-5]. Although adjuvant chemotherapy modestly improves the disease free and overall survival of patients with Dukes stage C colorectal cancer, there is a need for new adjuvant therapies that have both improved efficacy and fewer side effects. In this regard, the proven efficacy of adjuvant 17-1A therapy in a small group of patients with Dukes stage C colon cancer has shown that antibodies may have optimal efficacy when used in the setting of minimal residual disease [4]. Furthermore, there is a good rationale for the use of combinations of antibodies, or indeed for their use with traditional chemotherapy, so as to deliver a range of potentially synergistic anti-tumor activities [6-8].

The murine monoclonal antibody 30.6 recognizes an antigen that is expressed on colorectal carcinomas and their metastases. Expression of the antigen is greatest on well differentiated colorectal tumors, is less pronounced on poorly differentiated tumors, and is usually absent from most undifferentiated carcinomas [9]. The biochemical nature of the 30.6 antigen has not been elucidated, but it is expressed only on the luminal surface of glandular cells, and is not released into the circulation. The antigen is found in gastrointestinal epithelium, as well as in pancreatic acini, hepatocytes, alveolar pneumocytes and prostatic acinar epithelium. However, it is not expressed in other organs or tissues of the urogenital tract or central nervous system [9]. The murine 30.6 antibody has been shown to localize to subcutaneous human colorectal cancer xenografts in nude mice [10], as well as

to primary and secondary tumor deposits in patients with metastatic colorectal cancer [10,11]. Furthermore, the antibody, whether used alone, radiolabelled, or conjugated to cytotoxic drugs, is able to strongly inhibit the growth of human colorectal carcinoma xenografts in nude mice [12]. Phase I clinical studies of N-acetylmelphalan coupled to 30.6 showed that patients produced a human anti-mouse antibody response (HAMA) that precluded further dosage escalation [13,14].

The development of HAMA could impair the therapeutic effectiveness of 30.6, either by interfering with its binding to antigen, or by reducing its bioavailability. Replacing the murine constant regions with a human γ1 region was likely to reduce the immunogenicity of the antibody and also maximize its effector function. Mount et al., described a chimeric version of this antibody (c30.6) that binds with moderate affinity (Ka = 1×10^8 M-1) to its antigen, and can mediate in vitro antibody-dependent cell mediated cytotoxicity (ADCC) [15]. While this chimeric antibody was not able to lyse cells in the presence of either human or rabbit complement, it had anti-tumor activity in SCID mice bearing subcutaneous human colorectal cancer xenografts. A 40% reduction in tumor size was observed after intraperitoneal c30.6 administration, with maximal anti-tumor activity while the c30.6 antibody was being administered.

The chimerised 30.6 antibody therefore has a number of characteristics, which make it an attractive antibody for further clinical development. These include its ability to induce ADCC, its pattern of tissue reactivity, affinity, potential for reduced immunogenicity, and documented anti-tumor activity in animal models. We report here the results of single dose escalation studies of the chimeric 30.6 monoclonal antibody in patients with metastatic colorectal cancer. The primary objectives of the study were to evaluate safety, pharmacokinetics, and biodistribution of the c30.6 antibody in this patient population.

SUMMARY OF THE PRESENT INVENTION

The present inventors have found, however, that the administration of the 30.6 antibody leads to adverse side effects. The present inventors have found that these side effects may be ameliorated or prevented by pre-administration of H1 and/or H2 receptor antagonists.

ART 34 AMDT

Accordingly, in a first aspect the present invention provides a method of ameliorating or preventing temporal progression of burning cutaneous erythema in a subject wherein the erythema progresses successfully from the face to the chest, genitalia, palms and soles of the subject which method comprises administering to a subject in need thereof an effective amount of an H1 and/or H2 receptor antagonist.

In a preferred embodiment of the first aspect, the temporal progression of burning cutaneous erythema is caused by administration of an antibody.

In a second aspect the present invention provides a method of treating colorectal carcinoma in a subject, the method comprising administering to the subject 30.6 antibody and an amount of an H1 and/or H2 receptor antagonist effective in reducing at least one adverse side effect associated with administration of an H1 and/or H2 receptor antagonist.

In a third aspect the present invention provides a method of ameliorating or preventing at least one adverse side effect associated with the administration of 30.6 antibody to a subject, the method comprising administering an effective amount of an H1 and/or H2 receptor antagonist to the subject in conjunction with administration of the 30.6 antibody.

In a preferred embodiment of the first and second aspects of the present invention, the method comprises administering an H1 and H2 receptor antagonist.

In a preferred embodiment of the present invention, the H1 and/or H2 receptor antagonist is a non-specific antagonist. By 'non-specific' we mean that the H1 or H2 receptor antagonist interferes with the activity of at least one other histamine receptor. Using non-specific H1 and H2 antagonists, it is possible to administer to the subject a combination of antagonists which effectively interferes with or blocks the activity of all histamine receptors (eg. the H1, H2 and H3 receptors).

In a further preferred embodiment of the present invention the H1 receptor antagonist is selected from the group consisting of promethazine, pheniramine, trimeprazine, methdilazine, cyproheptadine, dexchlorpheniramine, fexofenadine, pseudoephidrine, azatidine, cetirizine and pharmaceutically acceptable salts thereof. Preferably, the H1 receptor agonist is promethazine or a pharmaceutically acceptable salt thereof.

In a further preferred embodiment, the H2 receptor antagonist is ranitidine or a pharmaceutically acceptable salt thereof.

In a further preferred embodiment, the H1 and/or H2 receptor antagonists is administered to the subject prior to administration of 30.6 antibody. Preferably the antagonists are administered at least one hour prior to 30.6 antibody administration.

The H1 and/or H2 receptor antagonists may be administered by any suitable mode. For example, the mode of administration may be oral, intravenous, intramuscular, intraperitoneal, transdermal, intradermal, transcutaneous, and all other routes known in the art including inhalation. In a preferred embodiment, the H1 receptor antagonist is administered intramuscularly. In a further preferred embodiment, the H2 receptor antagonist is administered intravenously.

In yet a further preferred embodiment, the H1 and/or H2 receptor antagonist is administered in large doses. Preferably, the dose level of the H1 and/or H2 receptor antagonist is around the maximum tolerated dose. More preferably, the dose level of the H1 receptor antagonist is around 50 - 70 mg and the dose level of the H2 receptor antagonist is around 50 mg.

In one preferred embodiment, the 30.6 antibody is a chimeric 30.6 antibody. Preferably, the chimeric 30.6 antibody comprises human constant regions.

In another preferred embodiment, the 30.6 antibody is a murine antibody.

In another preferred embodiment, the 30.6 antibody is a humanized antibody.

When used herein the term "antibody" should be construed as covering any specific binding substance having a binding domain with the required specificity. Thus, the term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide including an immunoglobulin binding domain, whether natural or synthetic. Chimeric molecules including an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The disclosure of all references referred to in this specification are included herein by cross-reference.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Pharmacokinetic analysis of serum from patients treated with unlabelled c30.6 at doses of 10-50mg/m^2. An ELISA was used to determine the concentration of c30.6 in the serum (see pateients and methods section).

DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting examples.

Example 1: Clinical trial of the chimeric monoclonal antibody c30.6 in patients with metastaatic colorectal cancer

The primary objective of this clinical trial was to evaluate the safety and immune responses to intravenously administered chimeric 30.6 antibody (c30.6) in patients with metastatic colorectal cancer.

The secondary objectives were to

- Determine the pharmacokinetics, tissue distribution and imaging characteristics of the antibody so as to determine the optimal biologic dose.
- Determine the efficacy of the antibody in patients with metastatic cancer.

Patients and Methods

Production of c30.6

Recombinant c30.6 (IgG1, kappa) antibody is secreted from Chinese Hamster Ovary Cells (CHO). The cloned 30.6 antibody heavy and light chain variable region cDNAs (obtained from the Austin Research Institute, Melbourne) were sub-cloned into the antibody expression vectors pG1D102 and pKN100 (Medical Research Council, Cambridge, UK). The vectors containing heavy and light chains were transfected into the host cell line CHO DG44 (from Dr Larry Chasin, Columbia University, NY). Following selection and screening, the production cell line 10A75H2.2F5 was isolated. The master cell bank of 10A75H2.2F5 was found to be free of microbial contaminants, murine adventitious virus and retrovirus. As expected, transmission electron microscopy demonstrated the presence of endogenous retrovirus particles in the CHO cells. c30.6 antibody was manufactured using

batch fermentation with serum free media, and was purified via a multi step procedure incorporating five chromatography and two viral inactivation/removal steps. The antibody was separated by Protein A affinity chromatography and then treated with solvent/detergent to inactivate lipid-enveloped viruses. Further purification by anion and cation exchange chromatography removed residual proteins, solvent/detergents and nucleic acids. The purified c30.6 was further purified and formulated into 0.9% saline using two gel filtration columns. Finally, the formulated bulk was sterile- and viral-filtered prior to dispensing into Hypak glass syringes (Becton Dickinson, NJ). Stability studies showed that the c30.6 antibody was stable in this formulation for at least sixteen months. Purity, identity, activity and safety were confirmed prior to release of the vialed product for clinical use.

Radiolabelling of c30.6

The c30.6 antibody (5 mg in 2 ml) was labelled with ^{123}I (2 GBq in 1 ml; ARI, Sydney) using the Iodogen method and the labelled antibody was purified by gel filtration using a Superose 12 HR column (Pharmacia, Uppsala, Sweden) coupled with FPLC. Prior to purification, the iodine incorporation was 68.0 ± 7.0%. The immunoreactive fraction of the labelled antibody was estimated by the Lindmo method [16] using the human colorectal cell line HT-29. The average immunoreactivity of the radiolabelled preparations was 60.7 ± 8.6%. The radiolabelling method was validated to produce sterile and pyrogen free product. Serum samples from patients injected with radiolabelled antibody were analysed by gel filtration chromatography for the presence of aggregates and free iodide using a Superose 12 HR10/30 gel filtration column.

Patient selection

For entry to the study, the patients were required to have histologically proven metastatic colorectal cancer, and to have adequate renal (creatinine <125% of the upper limit of the normal range), hepatic (bilirubin <125% of the upper limit of the normal range, prothrombin time < 1.3 times control) and haematological function (white blood count >4.0 x 10^9/L, platelet count >100 x 10^9/L, haemoglobin >100 gm/L) as well as the presence of measurable metastatic disease (at least one site 1 cm or greater). Each patient was also required to have a WHO performance status of less than 2 and a life expectancy of at least 3 months. Patients were excluded where they had

undergone chemotherapy or radiotherapy in the preceding four weeks or who had received immunosuppressive therapy in the preceding three months.

The 30.6 antigen is not detectable in paraffin embedded tissues, but is expressed in almost all moderately and poorly differentiated colorectal carcinomas [8] and therefore documentation of antigen expression was not required for entry into the study. Patients with severe non-malignant systemic disease or who were HIV-positive or had uncontrolled infection were precluded from entry. Those individuals who had previously been exposed to murine or chimeric antibody or antibody fragments were also excluded from entry. Detailed informed consent was obtained from all patients in accordance with the St Vincent's Hospital Human Ethics committee.

Clinical trial design

The first four patients entered in this study received 3 mg of ^{123}I-c30.6 (30 mCi). The next thirteen patients received a single dose of antibody at doses of 10 (five patients), 25 (five patients), and 50 mg/m^2 (three patients). Immediately following the infusion of unlabelled antibody, six of the thirteen patients also received 3mg of ^{123}I–c30.6 antibody. Patients were selected for administration of ^{123}I–c30.6 based on the site and extent of their disease, as well as performance status. Those individuals who received radiolabelled antibody were treated with Lugols iodine for three days before and two days after the antibody infusion.

Study measurements

All adverse events that occurred within 28 days of administration of the antibody were recorded and graded according to the Southwest Oncology Group Criteria. Measurement of hematology, serum chemistry, liver function tests, complement, human anti-c30.6 (HACA) and serum c30.6 levels were performed at regular intervals for a period of 6 months after the treatment. Physical examination, serum CEA levels, CT and PET imaging studies were performed at four to six weeks after treatment to assess the tumor response. A partial response was defined as a decrease of greater than 50% in the total sum of the products of the bidimensional measurements. Complete remission was defined as the disappearance of all disease, while stable disease was defined as no significant change in tumor measurements. A single expert (GB-W) who was unaware of the treatment protocol examined all imaging studies.

Pharmacokinetics of c30.6

To determine the pharmacokinetics of c30.6, serial blood samples were assayed by either gamma counting to detect radiolabelled c30.6 (used in the first four study patients) or ELISA to detect both unlabelled and radiolabelled antibody (used in all other study patients). In each patient who received radiolabelled antibody, EDTA treated blood samples were collected at 2, 4, 8, 15, 30 minutes and 1, 4, 24 and 48 hours. After 48 hours 1 ml plasma samples were counted on a gamma counter (Packard Instruments, autogamma 5650, IL). The total administered dose was determined using a dose calibrator and the dpm calculated by correcting for the efficiency of the gamma counting.

Serum c30.6 levels were determined using a competitive, solid-phase enzyme immunoassay, in which chimeric antibody in serum competes with exogenous biotin-labelled c30.6 for binding to sheep anti-c30.6 antibodies coated onto ELISA plates. Plates (Nunc-Immuno MaxiSorp) were incubated overnight at 4°C with affinity purified sheep anti-c30.6 antibody diluted 1:200 in coating buffer (100 mM sodium bicarbonate, 0.1% Bronidox pH 8.5). Following two washes in PBS/1% Tween/0.1% Bronidox the plate was blocked with PBS/1% skim milk/0.5% BSA/0.1% Bronidox for 90 minutes at 37°C.

Patient serum samples were collected prior to the infusion then at 1, 4, 24 and 48 hours and again at 6, 8 and 15 days. Samples and standards were heat inactivated (60°C, 30 minutes), diluted in pooled heat inactivated human serum and then mixed with an equal volume of biotinylated c30.6 (1:2000 in blocking buffer diluted in PBS/0.1% skim milk/0.05% BSA/0.1% Bronidox). This mixture was incubated on the washed plate at 37°C for 2 hours, washed, then incubated with alkaline phosphatase-streptavidin (Jackson, USA, 1:2000 in sample diluent) for 60 minutes at 37°C. Following further washes, p-nitrophenyl phosphate in carbonate buffer was added and the plate was incubated at 37°C until the lowest point on the standard curve (1 ng/mL c30.6) had an absorbance at 410 nm of 2.0 (Dynatech MR 7000 Microplate Reader). Each assay included a set of standards of c30.6 (2-1000 ng/ml) diluted in pooled human serum and mixed with biotinylated c30.6. Standard curves and serum antibody concentrations were calculated using the Assay-Zap assay program (Elsevier-Biosoft, Cambridge, UK). The detection limit of the assay was 20 ng/ml although c30.6 levels of 1 ng/ml

were detectable. Pharmacokinetic data analysis was performed using the non-linear parameter estimation application Minim (Dr Robert Purves, Pharmacology, University of Otago Medical School, NZ).

Measurement of human anti-c30.6 (HACA) levels

Human anti-30.6 levels were determined using a bridging ELISA. In this assay, serum human anti-c30.6 antibodies act as a bridge between c30.6 antibody coated on an ELISA plate and biotinylated c30.6 (sulfo-NHS-LC-biotin, Pierce, USA) in solution. A signal therefore indicates that biotin has been linked to the solid phase and that anti-c30.6 antibodies were in the serum sample. Plates were coated with c30.6 for 16 hours at 4°C (2 µg/ml in coating buffer) and blocked as described above. Following the addition of biotinylated c30.6, patient serum samples were added to the plate. Each plate also included the following controls; goat anti-human IgG Fcγ antibody (0.1 – 1000 ng/ml; Jackson Immunoresearch) for quantitation of the HACA response, sheep anti-c30.6 as a positive control and patient baseline serum samples as negative controls. Alkaline phosphatase-streptavidin was added to washed plates, and colour was developed and analysed as described above. The limit of detection of this assay was 2 ng/ml of goat anti human IgG Fcγ.

Imaging and dosimetry studies

In those patients who received ^{123}I-c30.6, anterior and posterior whole body planar images (256 x 1024 matrix, 15 minutes/meter) were performed immediately and at 1, 24 and 48 hours after injection. In addition SPECT images (128 x 128 matrix, elliptical orbit, 64 views, 360°, 30 sec/view) of the chest and abdomen were obtained at 24 and 48 hours. A GE XRT gamma camera and high resolution low energy collimator was used for all imaging. Radiation dosimetry was estimated by using the whole body images from 0, 4, 24 and 48 hours post injection. The cumulated activity was calculated for various organs of interest and then converted into residence times. These times were entered in to the MIRDOSE program (Oak Ridge National Laboratories, USA) to determine the internal radiation dosimetry.

Results

Patient characteristics

The characteristics of the 17 patients with progressive metastatic colorectal cancer (7 rectal, 10 colon) entered this study are shown in Table 1, along with a summary of prior therapies and sites of disease at the time of

Table 1

Patient ID	Patient No/Sex Age	Dose mg (*)	Primary site	Primary resected	Dukes D at presentation	Prior surgery (χ)	Prior radio therapy (χ)	Prior chemotherapy (χ)	Sites of disease (ϕ) (size range)	Response determined by CT/PET scan
1	37,M	*2.1	SC	Y	Y	LAR (3 wks)	Nil	Nil	Multiple bilobar hepatic	N/A withdrew from study 8 lesions P, 2NC
2	70,M	*3.1	DC	Y	N	LH (3 yrs)	Nil	Adjuvant 5FU/FA (3yrs)	Left cervical lymph node, hepatic (10-40mm), pulmonary, mesentery, right adrenal	
3	57,M	*3.2	Rectum	Y	N	LAR (3 yrs)	Nil	Nil	1 right hepatic lobe (20mm), nodes all porta hepatis & around duodenum	2 new lesions, 2P, 2NC
4	58,M	*3.1	TC	Y	Y	SC (0 mths)	Nil	Nil	Bilobar hepatic (10-15 mm) and left femur	2 new lesions, 4P, 2NC
5	67,M	17	Rectum	Y	N	AR (4 yrs)	Adjuvant pelvis (4 yrs)	Adjuvant 5FU/FA (4 yrs)	2 hepatic mets, 8 & 60mm	2 progressed
6	52,M	17 & *3.9	AC	Y	N	RH (0 mths)	Nil	Adjuvant 5FU/FA (2 mths)	Right hepatic metastases (20-50mm)	ND (Rapid progression, died at 8 weeks)
7	71,F	13.8 & *3.0	Rectum	N	Y	ND	Nil	Nil	Bilobar hepatic metastases (10-20 mm), rectal tumour infiltrating prerectal space	

Substitute Sheet (Rule 26) RO/AU

8	60,M	10 & 13.0	Rectum	Y	N	AR (5 yrs); AP (4yrs)	Palliative pelvis (2 yrs): Brachy therapy (2 yrs); Palliative left iliac crest (8 mths)	Palliative 1A, 5FU/MitC (18 mths): palliative IV 5FU/MitC/FA (8 mths); Camptosar (8 wks)	Bony, pelvis, pulmonary	On CT, NC, On PET, progression in lung but response in pelvis
9	72,M	13.0	Rectum	Y	N	AR (4.5 yrs)	Adjuvant pelvis (4.5 yrs)	Adjuvant 5FU/FA (4 yrs)	Right lobum, para-aortic lymph nodns, hepatic	ND Refused follow up CT
10	69,M	40	Rectum	Y	N	LAR (6 yrs)	Palliative, local recurrence (3.5 yrs)	Palliative, radiosensitiser 5FU (3.5 yrs)	Left hepatic, pelvis, pulmonary (10-40mm)	1 new pulmonary lesion, 2 P pulmonary
11	60,M	45 & 13.0	DC	N	Y	ND	Nil	Nil	Bilobar hepatic metastases (5-30mm), descending colon tumour	Slight progression hepatic lesions, primary smaller
12	75,M	47.5	Sigmoid	Y	N	PC (10 mths)	Nil	Nil	Bilobar hepatic, Bony L2, T10, left iliac crest	ND (Died at 18 days, from AMI)
13	42,M	48 & 13.0	AG	Y	Y	RT (2 yrs)	Palliative mediastinum (3 mths)	Palliative 5FU/FA (18 mths); Palliative 5FR (3 mths)	Hepatic (up to 80mm), pulmonary (2mm), paraortic LNs (up to 20mm)	NC in 4 measurable lesions, lung, liver, lymph nodes Differential response on PET
14	73,M	47.5	SC	Y	N	AR (18 mths)	Nil	Adjuvant 5FU/FA (1 yr)	Hepatic (2-30mm)	2 lesions, P, 2NC

15	50,M	05 & *3.0	AC	Y	N	RH (4 yrs): Local wound mot (6 mths)	Nil	Palliative 5FU (3 mths)	Bilobar hepatic and a nodal disease	CT, ND Suggestion of early response on PET
16	70,M	05	Hepatic flexure	Y	Y	RH (3 mths)	NB	NB	Bilobar hepatic metastases (15-30mm), portocaval node (20mm)	2 hepatic lesions P, 2NC; lymph node NC
17	63,M	00	Rectum	Y	Y	AP (4 mths)	Adjuvant pelvic (2 mths)	Radiosensitiser 5FU (2mths)	Bilobar hepatic metastases, 5-20mm	CT, ND Progression on PET

Abbreviations: *mg radiolabelled c30.6 administered after the unlabelled antibody. SC, sigmoid colon; TC, transverse colon; AC, ascending colon; HF, hepatic flexure; DC, descending colon. Time since last treatment (χ). Prior surgery. AP, abdominoperineal resection; RH, right hemicolectomy; PC, proctocolectomy; AR, anterior resection; LAR, low anterior resection; SC, subtotal colectomy; LH, left hemicolectomy; ND, not done. Chemotherapy, 5FU, 5Fluorouracil; FA, Folinic acid; IA intraarterial; IV intravenous; Mito, Mitomycin C; (ϕ) sites of disease at the time of treatment with antibody. Response, NA not accessable; NC, no change; P progressed.

administration of c30.6. The participants were aged from 37 to 78 years, and the sites of their metastatic disease included the liver (16), lungs (5), mesentery (2), lymph nodes (5), bones (3), adrenal (1), and pelvis (2). Seven individuals had initially presented with metastatic disease, and in two of these cases the primary tumor had not been resected prior to antibody administration. Of the 10 individuals without metastatic disease at initial presentation, five had received adjuvant 5-Fluorouracil based chemotherapy shortly after resection of their primary tumor, and one of these individuals developed metastatic disease while undergoing adjuvant therapy.

At the time of antibody therapy, chemotherapy was considered inappropriate in five patients, either because of progressive disease despite palliative therapy (3), relapse on adjuvant therapy (1) or refusal to undertake palliative therapy (1). A further 12 individuals had progressive disease but were as yet either asymptomatic, or their symptoms were well controlled with other medications.

Antibody infusions

The unlabelled c30.6 antibody was administered in an outpatient clinic by infusion through a peripheral line. Dose levels of 10, 25, and 50 mg/m^2 were diluted in 50, 100 or 500 mls of 0.9% sodium chloride and delivered over 40, 60 or 120 minutes respectively. Ten individuals were injected with a slow intravenous push of 123I-30.6 (30mCi), either alone (patient ID number 1-4) or immediately following the completion of the unlabelled antibody infusion (patients ID 6-8, 11, 13, 15, Table 1). The first seven patients (ID 1-7) did not receive a premedication prior to the treatment, while the next seven (ID 8-14) were premedicated with a combination of Loratidine 5-10 mg and paracetamol (500mg) up to one hour before antibody administration. The final three patients (ID 15-17) were premedicated with promethazine (50-75mg im) two hours prior to antibody infusion, and with Ranitidine (50 mg intravenously) one hour before treatment.

Antibody toxicity

All but one patient completed the scheduled infusion of antibody. In this patient the last two of 48mg of antibody was not administered because he experienced uncontrolled, severe burning erythema of his face, palms, soles and genitalia. A total of fifty-six adverse events occurred in the first 24 hours after infusion of antibody, and of these fifty-five were directly attributable to the antibody infusion (Table 2). The most frequent side effect

Table 2

Side effect	Radio-labelled (n=4)	Dose in mg/m² (patients per group) 10 (n=5)	25 (n=5)	50 (n=3)
Generalised				
• Back pain	0	0	1	0
• Headache	1	0	0	0
• Rigors and/or temperature	1	0	2	1
• Shoulder/hip pain	0	1	0	1
Gastrointestinal				
• Abdominal pain	0	0	0	3
• Nausea	0	1	1	0
• Nausea & vomiting	0	1	0	2
Respiratory				
• Breathless	0	0	1	0
• Sneezing	0	0	1	2
Mucocutaneous				
• Burning erythema (eyes, external auditory canal, nasal mucosa)	0	3 (2ML, 1S)	4 (1ML, 2MD, 1S)	2 Mild
• Burning erythema palms, soles	0	3 (1ML, 2MD)	4 (1ML, 2MD, 1S)	2 ML
• Burning (genital)	0	2 (1ML, 1MD)	2 (1ML,1 S)	1 Mild
• Burning erythema (Face, ears)	0	2 (1MD, 1S)	3 (1mild, 1MD,1 S)	2 Mild
• Rash	0	0	0	1 ML
Tumour pain				
• Chest	0	1	0	0
• Right hip	0	0	1	0
• Hepatic	0	0	1	0
• Sciatica	0	1	0	0
Total Adverse Events	**2**	**15**	**21**	**17**

Acute adverse events related to infusion of the c30.6 antibody.

An acute event was defined as a side effect occurring within 24 hours after treatment with the antibody. Those events which were either definitely or possibly a consequence of antibody administration are included in the table. Abbreviations: ML mild, MD moderate, S severe.

(56% of all adverse events) was a constellation of symptoms consisting of severe burning and erythema of the face, chest, neck, ears, palms, soles, and genitalia (penis, testis, vagina, external labia). This reaction was often accompanied by conjunctival injection, itching of the external auditory canal, injection of nasal mucosa, stuffy nose, and discomfort or burning around the lips and throat. These symptoms typically began 30-300 minutes after the infusion commenced (median 70 minutes) and lasted from 5 minutes to 4.3 hours (median 1.5 hours). Although each patient did not invariably experience burning erythema at each site, the temporal involvement of the skin typically followed the following sequence: face/genitalia, palms then soles. These symptoms were self-limiting and did not require admission to hospital yet in some patients they were of such severity as to require narcotic analgesia. Once these symptoms had begun, they were refractory to all modalities of treatment including cold packs, drugs such as H1 blockers (promethazine, 25-50 mg intravenously), hydrocortisone (up to 200 mg iv), sudafed 60 mg po, paracetamol, and narcotics. At the dose level of 10 mg/m^2 all five patients experienced these mucocutaneous side effects and of the ten events, two were mild, six moderate and two severe. At the 25 mg/m^2 level, four of the five patients demonstrated this side effect with 4 mild, 5 moderate, and 4 severe reactions.

These reactions occurred despite pre-treatment in some cases with Loratidine and paracetamol one hour prior to infusion (two of the five individuals at the 10 mg/m^2 dose level and all individuals at the 25 mg/m^2 dose level). At the dose level of 50 mg/m^2 pre-medication with promethazine and Ranitidine was given to all three patients and resulted in a dramatic improvement in the severity of this syndrome with all events being reported as mild.

In an attempt to further elucidate the nature of this mucocutaneous reaction, tryptase was measured in serum samples collected at baseline, 1 hour and 8 day time points from a number of patients (ID 6, 8, 13, 15) including individual 13 who had demonstrated the most florid reaction. None of these samples demonstrated a significant rise in tryptase at the one hour time point. Similarly, 1 hour serum and 24 hour urinary histamine and methylhistamine levels were normal in a subset of three individuals.

At the highest dose level, one individual (patient ID 17) experienced mild abdominal pain and vomiting one hour after the commencement of the

infusion which lasted for 4 hours. Analgesics were not required, as the symptoms were mild. The clinical examination was unremarkable and serum amylase was 73U/L (normal <100U/L). The following day, this patient developed a further episode of mild abdominal pain associated with two episodes of vomiting. On this occasion the amylase was significantly elevated at 1030 U/L, as was the serum lipase 660 U/L (normal <60 U/L), consistent with the diagnosis of acute pancreatitis. The symptoms rapidly resolved without any treatment and the amylase returned to normal within 48 hours. Amylase levels on stored serum samples from six other patients (ID 11-16) were found to be normal.

All other adverse events were grade 1 except one instance of grade 2 tumor pain (sciatica), and one instance of rigors (patient ID 11). The following events occurred up to 28 days after antibody administration but were not thought to be related to antibody administration; death from an acute myocardial infarct at 18 days and two instances of a dry throat and mouth secondary to promethazine. The following events were probably related to antibody administration; gritty eyes for 4 days, vomiting and nausea for 2 days after infusion (two patients, grade 2), and anorexia (2 days).

FBC/Biochemistry/LFTs, complement

No clinically significant changes in hematology, biochemistry, or complement occurred in the 4 week period following antibody administration. There was considerable inter-patient variability in the liver function tests measured in the first 28 days. For instance, the alkaline phosphatase fell by 50% in some individuals while in others increased to 30% above baseline. Similar changes were noted in γ-glutamyltransferase and the transaminases. Overall there was no significant change in liver function tests which depended on patient dose, predicted response or was discordant with the pattern of their liver function tests in the subsequent 3 months.

Pharmacokinetics

Pharmacokinetic analysis of plasma ^{123}I-c30.6 levels showed a biexponential clearance pattern with an α half-life of 2.5 + 0.7 minutes (mean + SD) and a β-half-life of 46 + 15 hours. An ELISA was used to determine the serum c30.6 concentrations in those patients treated at the 10, 25 and 50 mg/m2 dose level (Figure 1). At the 10 mg/m^2 dose the β half-life was 51 + 5 hours and this increased to 57 + 19 hours at 25 mg/m^2 dose level

and 81 + 15 hours at 50 mg/m^2. The maximum observed serum concentration increased from 0.97 µg/ml at the 10 mg level, to 2.4 µg/ml at 25 mg and 7.9 µg/ml at 50 mg/m^2. Importantly, at the 50 mg/m^2 dose level, the serum concentration of c30.6 remained at approximately 100 ng/ml from one week to at least three weeks after the infusion. It is also of note that there was marked inter-patient variability in all pharmacokinetic parameters.

HACA

Serum samples were assayed for HACA at 2, and 6 weeks as well as 3 and 6 months. One of 17 patients manifested a HACA response and this was detectable at 14 days to 6 months post treatment. This patient only received 2.1 mg of ^{123}I-c30.6 and was not treated with any unlabelled antibody. The level of HACA was low (14 ng/ml at three and six months), and could only be detected in neat serum. In comparison, sheep immunized with c30.6 have anti-c30.6 levels of approximately 400 µg/ml in this assay.

Tumor response

While all seventeen patients had measurable disease, tumor response was only assessable in 13 of these individuals (Table 1). Two patients died before imaging could be undertaken (one from a myocardial infarct and the second from progressive disease) and a further two patients refused follow up scans (one withdrew from study and the other remained on study but did not undergo imaging). There were no partial or complete responses in the ten assessable patients who received unlabelled c30.6, with or without ^{123}I-c30.6. At 4 – 6 weeks, five patients had progressive disease and two had stable disease. The remaining three patients (ID 8, 13, and 15) had evidence of improvement in at least some of their metastatic lesions. For instance, in patient 8 there was no change in the CT scan performed at 7 weeks, however the PET scan demonstrated a 22-30% reduction in glucose metabolism at measurable sites within the extensive pelvic disease. The PET scan also showed progressive disease in the chest with an increase in the size and avidity of lesions at base of right lung, hilum and mediastinum. This progression was not noted on CT but subsequently became readily apparent. PET scanning of patient 13 showed that a qualitative decrease in glucose avidity of some hepatic lesions was readily apparent at 7 days, with a further decrease at 6 weeks. The 6 week scan also identified new lesions particularly in the chest. These new lesions were also not detected on CT. Patient 15 refused CT scanning, but a PET scan performed at day 13 demonstrated a 7%

decrease in glucose uptake in the extensive intra-abdominal disease. By 7 weeks, this patient had deteriorated clinically, and PET scanning confirmed progressive disease with a 24-35% increase in glucose uptake.

The median percentage change in CEA from baseline to 8 weeks was found to be +11% (range –20 to +169), 0% (range –38 to + 132) and +6% (range 0- +54%) at the 10, 25 and 50 mg/m2 dose levels. All patients in whom PET scanning showed some reduction in particular sites of disease were also found to have small increases in serum CEA levels, probably reflecting the previously mentioned occurrence of progression at other sites. Other patients such as patient ID 10. demonstrated a sustained fall in CEA from 195 at baseline to 120 µg/L at 8 weeks (normal <3.0) yet CT scanning clearly showed progression of disease.

Biodistribution and imaging

Gel filtration chromatography was used to analyse serum collected at 4, 24 and 48 h from two patients who received only 123I-c30.6. The radioactivity from all samples eluted as a single peak at a molecular weight of 160 Kda, indicating that there was no detectable complexing or breakdown of the labelled antibody in-vivo. Further evidence supporting the *in vivo* stability of the antibody and label was provided by analysis of 48 hour urine collections which showed that 17-21% of the injected activity was excreted in this period.

Whole body planar images obtained at 0, 4, 24 and 48 hours post-injection indicated that there was a very rapid and intense uptake of activity in the liver, coupled with a decrease in the blood pool activity over the first hours. At the zero time point, planar images of the four patients injected with ^{123}I-c30.6 (patient ID 1-4, Table 1) showed that 50% (range = 43-54%) of the dose localized in the liver and at 48 hours the localization remained high at 44% (range =35-50%). However, as expected, the pre-infusion of unlabelled c30.6 significantly reduced the hepatic uptake of ^{123}I-c30.6 (mean hepatic localization 25%, range 21-35% at the zero time point). In patients 1-4 the mean whole body effective dose equivalent was $3.19 \pm 0.37 \times 10^{-2}$ mGy/MBq.

Single photon emission computed tomography (SPECT) obtained at both 24 and 48 hours post ^{123}I-c30.6 injection identified primary and some metastatic lesions. The two primary tumors (patient ID, 7 and 11) which had not been resected prior to study were successfully imaged as hot lesions with radioactivities of 1.2 and 2% . Hepatic lesions were visualized either as hot

lesions, cold lesions or as regions of low activity surrounded by a hot rim. Although all individuals with hepatic disease were reported to have abnormal areas of hepatic uptake SPECT did not identify all the lesions reported on CT and PET scanning. As expected the pattern of uptake was somewhat dependent on the size of the lesion. This observation is consistent with previous reports in which lesions with a diameter of greater than 2.5 cm usually appeared as cold defects with or without a hot rim, while lesions 2.0 cm or less tended to show high uptake of radioactivity 17. Although two patients with extra-abdominal disease (patient ID 2 and 13) participated in the study, SPECT imaging did not identify the pulmonary and nodal tumor sites in either case. However, one individual with extensive pelvic disease did demonstrate quite marked uptake of isotope at these sites (patient ID 8).

Discussion

In this study we describe the generation of a novel chimeric antibody and its clinical evaluation in individuals with metastatic colorectal cancer. In order to obtain additional data on biodistribution, pharmacokinetics and tumor targeting, we also treated a subset of patients with radiolabelled c30.6 antibody. ^{123}I was chosen for this purpose, since its short half life (13 hours) and low energy γ emission (159keV) allow for safe handling and improved imaging.

This study demonstrated that ^{123}I labelled c30.6 antibody was of insufficient specificity or sensitivity for use as a diagnostic imaging agent. While some lesions such as primary colorectal tumors were readily detected, other large tumor deposits in the lungs and lymph nodes remained occult. The detection of hepatic lesions was clearly disadvantaged by the combination of the high uptake of antibody in the liver and the short half-life of the label. Previous studies have demonstrated that this type of imaging problem can be overcome by the use of antibody fragments and alternative labels [18-20]. The whole body planar images graphically demonstrated the intensive and prolonged uptake of antibody into the normal liver parenchyma. Furthermore, it was shown that this non-specific uptake could be reduced by the pre-infusion of cold antibody. It is likely that non-specific hepatic uptake was one of the factors determining the antibody half-life and maximum serum concentrations. These parameters increased as the dose of infused antibody was escalated, probably as a result of saturation of hepatic

binding sites. The reservoir of antibody in the liver, and its slow release back into the circulation, may also account for the prolonged maintenance of serum c30.6 levels in patients who received doses of 50 mg/m^2. Despite the intensive hepatic uptake there was no biochemical or clinical evidence of hepatotoxicity.

The serum half life and maximum serum concentration seen with c30.6 (81 hours, 7.9 μg/ml at the 50 mg/m^2 dose) is comparable to that of other chimeric antibodies currently in clinical trial. For instance the half life of chimeric 17.1A was 100 hours and the maximum serum concentration was 11 μg/ml when used at a doses of 40 mg fortnightly for three infusions [21]. Similarly, the IDEC-C2B8 had a half life of 33 hours following a dose of 375 mg/m^2, and this increased to 76 hours after four infusions as a result of saturation of CD20 sites in the serum and circulation 1. On the basis of these and other studies, we anticipate that the half life and maximum serum concentration of c30.6 will increase following the infusion of multiple doses. Most side effects occurred within four hours of the antibody infusion, were self limiting and did not require admission to hospital. Some of these side effects such as rigors and headache have been frequently observed with other antibody therapies [3,22,23]. The gastrointestinal side effects, and in particular the episodes of nausea and vomiting, were also predictable, since c30.6 extensively cross reacts with the gastric mucosa.

One patient who received the highest dose of c30.6 developed an antibody-induced pancreatitis. Once again the symptoms were mild and self-limiting, and were disproportionate to the degree of elevation of serum amylase that was observed. It is probable that the pancreatitis resulted from binding of antibody to pancreatic structures. This may have taken the form of direct cytolysis of acinar cells, or obstruction secondary to cross-reactivity with ductal epithelium. Cross-reactivity against structures in the normal pancreas had previously been identified in immunohistochemical studies on post-mortem tissues, however precise localization of the 30.6 antigen was not possible due to the presence of extensive autolysis, as is typical of this tissue. Although the serum amlyase was normal in six other individuals, it appears that infusion of antibodies, like other drugs such as thiazide diuretics and azathioprine, can cause pancreatic injury [24].

The side effects observed with c30.6 are different to that generally seen with the administration of monoclonal antibodies. The table below lists

some of the side effects observed with the antibodies currently in use: Rituxan, Herceptin and Panorex.

	Incidence			
Adverse event	Herceptin [2] (patients = 352)	Rituxan [1] (patients = 315)	Panorex [34] (Patients = 83)	Panorex [35] (Patients = 490)
Cardiovascular				
Tachycardia	5		2	
Congestive heart failure	7	32	2	3%
Hypotension				
Haematological				
Anaemia	4			
Leucopaenia	3	33		
Neutropenia		21		<1%
Thrombocytopenia		25		
Gastrointestinal				
Nausea	33	55		10%
Diarrhoea	25		7	19%
Vomiting	23	23		5%
Nausea/Vomiting	8		4	
Anorexia	14			
Mucositis				2%
Abdominal pain	22	18	3	
Respiratory				
Increased cough	26			
Dyspnoea	22			
Rhinitis		25		
Bronchospasm		24		
Skin				
Rash		31		
Urticaria		24		
Pruritus		32		
Flushing			7	5%
Alopecia				1%

Adverse event	Herceptin [2] (patients = 352)	Rituxan [1] (patients = 315)	Panorex [34] (Patients = 83)	Panorex [35] (Patients = 490)
General				
Tumour pain	47		1	
Asthenia	42			8%
Fever	36	154	4	3%
Chills	32	102	4	4
Headache	26	43	2	2
Back pain	22			
Flu-like syndrome	10			
Allergic reaction	3		6	6

Skin reactions have previously been reported with the administration of antibodies. These reactions were usually mild, infrequent and qualitatively different to that observed in association with c30.6.

To our knowledge, the constellation of mucocutaneous symptoms seen in this study has not been described with other monoclonal antibodies, nor indeed with any other form of drug therapy. The temporal progression of burning cutaneous erythema involving at first the face, and then the chest, genitalia, palms and soles in succession, was particularly distinctive. Pain at these sites was often of such severity that it necessitated the use of narcotics. While there was some variability between individuals, it was apparent that the frequency and severity of this reaction increased proportionally to the dose of antibody administered.

Interestingly, this reaction occurred in the absence of symptoms classically associated with hypersensitivity reactions, such as bronchospasm, urticaria, facial and laryngeal edema, and hypotension. However, while the established reaction was refractory to all attempted therapies, it was readily prevented by premedication with high doses of combination H1 and/or H2 antagonists. The preference for high doses of these antagonists was recently illustrated in a follow up multi-dose trial of c30.6, where a 25% reduction in the dose of the H1 antagonist was associated with recurrence of this syndrome (unpublished observations).

A number of pathogenic mechanisms can be considered in regard to this mucocutaneous reaction syndrome. The possibility that the reaction was

the result of infusion of histamine contaminating the antibody preparations was excluded by retrospective analysis of all batches of antibody [25]. A more likely explanation was that the syndrome was the result of mast cell degranulation, either directly or indirectly mediated by antibody infusion. Mast cell degranulation causes the release of range of vasoactive and neuroactive mediators, such as histamine, tryptase and prostaglandins [26], all with pharamcological actions consistent with the vasodilation, pain and flushing of the skin seen following infusion. The protein tryptase is also released during mast cell degranulation, and serves as a useful marker of this event because of its long serum half life. However, serial estimations of tryptase and histamine in symptomatic individuals in this study failed to show elevation of serum levels. The possibility remains that mast cell degranulation may have occurred only at a local level. In this regard, elevated tryptase levels have been clearly demonstrated in bronchoalveolar and nasal lavage fluids from allergic individuals, despite the presence of unchanged normal serum levels [26-28].

Finally, it is possible that the reaction represents direct antibody cross-reactivity with the involved mucocutaneous tissues. The c30.6 antibody does show limited cross-reactivity with eccrine gland and duct epithelium, and its binding may have provided an indirect signal for mast cell degranulation. Whether the mechanism is a direct or indirect one, we postulate that the antibody induces a significant cutaneous mast cell degranulation, which is not associated with systemic effects.

The murine 30.6 was chimerised in order to reduce its immunogenecity and to enhance its cytotoxic effects. In a previous clinical trial of the murine antibody, all patients developed human anti-mouse antibodies [14] whereas only a single patient in the current study developed a HACA response. Importantly, this response was of a low level and in fact occurred in a patient who received only a low level of antibody. The lack of immunogenecity of c30.6 has allowed a multi-dose study to proceed, and early results indicate that no HACA occurs after the administration of four doses (unpublished observation).

The c30.6 antibody may kill tumor cells *in vivo* by at least two possible mechanisms. *In vitro* data indicates that it is capable of lysing human colorectal cells by antibody-dependent cellular cytotoxicity (ADCC), although it does not lyse the same cells in the presence of either rabbit or

human complement [15]. While the function the 30.6 antigen has not yet been defined, it is also possible that the binding of antibody could exert a direct cellular effect, in a manner similar to the effects of antibodies, which bind to the HER-2/neu receptor [29,30]. Despite these potential actions, there were no partial responses observed in this clinical trial. This finding is not dissimilar to that of many other early studies of single doses of monoclonal antibodies in the treatment of solid tumors [31-33].

In summary, the chimerised c30.6 antibody is not immunogenic in humans, and appears worthy of further study. It does however produce a unique profile of side effects, which although significant can be well controlled with appropriate pre-medication.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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ART 34 AMDT

CLAIMS:

1. A method of ameliorating or preventing temporal progression of burning cutaneous erythema in a subject wherein the erythema progresses successively from the face to the chest, genitalia, palms and soles of the subject which method comprises administering to a subject in need thereof an effective amount of an H1 and/or H2 receptor antagonist.

2. A method as claimed in claim 1 in which the temporal progression of burning cutaneous erythema is caused by the administration of an antibody.

3. A method of ameliorating or preventing at least one adverse side effect associated with the administration of 30.6 antibody to a subject, the method comprising administering an effective amount of an H1 and/or H2 receptor antagonist to the subject in conjunction with administration of the 30.6 antibody.

4. A method of treating colorectal carcinoma in a subject, the method comprising administering to the subject 30.6 antibody and an amount of an H1 and/or H2 receptor antagonist effective in reducing at least one adverse side effect associated with administration of the 30.6 antibody.

5. A method as claimed in any one of claims 1 to 4 in which the method comprises administering an H1 and H2 receptor antagonist.

6. A method as claimed in any one of claims 1 to 5 in which the H1 and/or H2 receptor antagonist is a non-specific antagonist.

7. A method as claimed in any one of claims 1 to 6 in which the H1 receptor antagonist is promethazine or a pharmaceutically acceptable salt thereof.

8. A method as claimed in any one of claims 1 to 7 in which the H2 receptor antagonist is ranitidine or a pharmaceutically acceptable salt thereof.

9. A method as claimed in any one of claims 3 to 8 in which the H1 and/or H2 receptor antagonist is administered to the subject prior to administration of 30.6 antibody.

10. A method as claimed in claim 9 in which the H1 and/or H2 receptor antagonist is administered at least one hour prior to 30.6 antibody administration.

11. A method as claimed in any one of claims 1 to 10 in which the H1 receptor antagonist is administered intramuscularly.

12. A method as claimed in any one of claims 1 to 11 in which the H2 receptor antagonist is administered intravenously.

13. A method as claimed in any one of claims 1 to 12 in which the H1 and/or H2 receptor antagonist is administered at a dosage level of around the maximum tolerated dose.

14. A method as claimed in claim 13 in which the H1 receptor antagonist is administered at a dosage of between 50 - 70 mg.

15. A method as claimed in claim 13 in which the H2 receptor antagonist is administered at a dosage of about 50 mg.

16. A method as claimed in any one of claims 3 to 15 in which the 30.6 antibody is a chimeric 30.6 antibody.

17. A method as claimed in claim 16 in which the chimeric 30.6 antibody comprises human constant regions.

18. A method as claimed in any one of claims 3 to 15 in which the 30.6 antibody is a murine antibody.

19. A method as claimed in any one of claims 3 to 15 in which the 30.6 antibody is a humanized antibody.

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9. A method as claimed in any one of claims 3 to 8 in which the H1 and/or H2 receptor antagonist is administered to the subject prior to administration of 30.6 antibody.

10. A method as claimed in claim 9 in which the H1 and/or H2 receptor antagonist is administered at least one hour prior to 30.6 antibody administration.

11. A method as claimed in any one of claims 1 to 10 in which the H1 receptor antagonist is administered intramuscularly.

12. A method as claimed in any one of claims 1 to 11 in which the H2 receptor antagonist is administered intravenously.

13. A method as claimed in any one of claims 1 to 12 in which the H1 and/or H2 receptor antagonist is administered at a dosage level of around the maximum tolerated dose.

14. A method as claimed in claim 13 in which the H1 receptor antagonist is administered at a dosage of between 50 - 70 mg.

15. A method as claimed in claim 13 in which the H2 receptor antagonist is administered at a dosage of about 50 mg.

16. A method as claimed in any one of claims 3 to 15 in which the 30.6 antibody is a chimeric 30.6 antibody.

17. A method as claimed in claim 16 in which the chimeric 30.6 antibody comprises human constant regions.

18. A method as claimed in any one of claims 3 to 15 in which the 30.6 antibody is a murine antibody.

19. A method as claimed in any one of claims 3 to 15 in which the 30.6 antibody is a humanized antibody.

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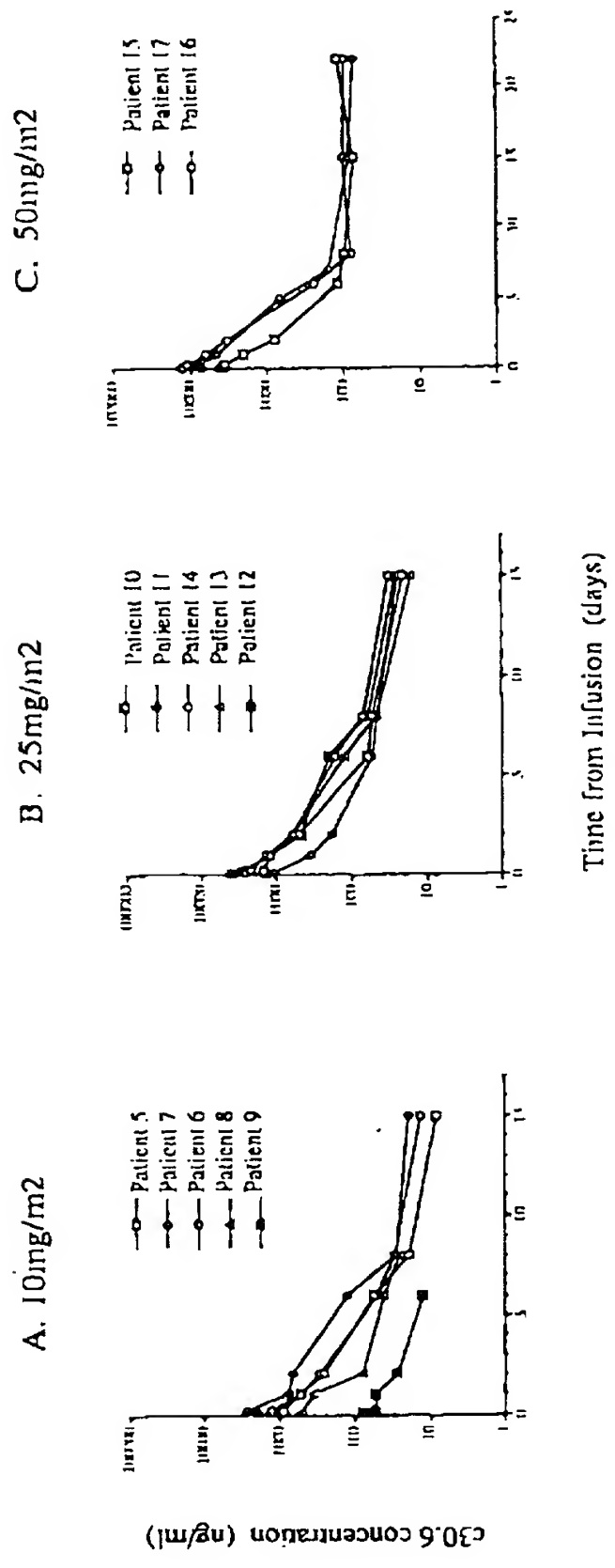

Figure 1

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(Domestic Non-Assigned/Foreign) Page 1

RULE 63 (37 C.F.R. 1.63)
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, mailing address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF TREATING CARCINOMA USING ANTIBODY THERAPY AND AMELIORATING SIDE EFFECTS ASSOCIATED WITH SUCH THERAPY

the specification of which (check applicable box(s)):

☐ is attached hereto
☒ was filed on December 7, 2001 as U.S. Application Serial No. Unassigned (Atty Dkt. No. 47-164)
☒ was filed as PCT International application No. PCT/AU00/00638 on 7 June 2000
and (if applicable to U.S. or PCT application) was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
PQ 0809	Australia	7 June 1999

☐ I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

☐ I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT International applications listed above or below:

Prior U.S./PCT Application(s): Application Serial No.	Day/Month/Year Filed	Status: patented pending, abandoned
PCT/AU00/00638	7 June 2000	

☐ I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And on behalf of the owner(s) hereof, I hereby appoint NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed), and the following attorneys thereof (of the same address) individually and collectively owner's/owners' attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Larry S. Nixon, 25640; Arthur R. Crawford, 25327; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Sr., 29366; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329; Joseph A. Rhoa, 37515; Raymond Y. Mah, 41426; Chris Comuntzis, 31097; Gary T. Tanigawa, 43180. I also authorize Nixon & Vanderhye to delete any attorney names/numbers no longer with the firm and to act and rely solely on instructions directly communicated from the person, assignee, attorney, firm, or other organization sending instructions to Nixon & Vanderhye on behalf of the owner(s).

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2-02

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